

Leaf epidermal characters of *Lonicera japonica* and *Lonicera confusa* and their ecology adaptation

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Abstract: The leaf epidermis of Japanese honeysuckle (*Lonicera japonica* Thunb.) and Wild Honeysuckle (*Lonicera confusa*) in the genus of *Flos Loniceræ* were mainly observed by scanning electron microscopes (SEM) to study the characteristics of stomata, trichomes and dermal cell, etc.. The results showed that stoma exists only on the lower epidermis and its distribution is irregular, and leaf epidermis consist of epidermis cells, stoma complexes and bushy trichomes including glandular hair and non-glandular hair. On the upper epidermis, anticlinal wall caves in sinuous groove to countercheck the transpiration. Evidences from leaf morphological structures serve as another proof on drought-resistant mechanisms. Some strumaes distributing regularly are hypothesized as oxalic calcium on the lower epidermis under laser scanning confocal microscopy (LSCM) with Fluo-3/AM, which can increase their endurance to drought stress. Therefore, the above characteristics of *Flos Loniceræ* can reduce the loss of water and make Japanese honeysuckle and Wild Honeysuckle adapt to the droughty environment at Karst area in southwest China. However, there is some difference of the two species. From the SEM (Scanning Electron Microscopy) result, it is shown that on the upper epidermis, some glandular hair regularly present along the midrib of Japanese honeysuckle, but Wild Honeysuckle has no glandular hair on the upper epidermis, which can verify the relationships of *Flos Loniceræ* species and provide the significance for classification of *Flos Loniceræ*.

Keywords: *Lonicera japonica*; *Lonicera confusa*; *Flos Loniceræ*; Dermal cell; Stomata; Trichomes; Ecology adaptation

Introduction

Japanese honeysuckle (*Lonicera japonica* Thunb.) and Wild Honeysuckle (*Lonicera confusa*) in the genus of *Flos Loniceræ* with developed rootstalks not only can live in the lean soil, but also can retain water and soil at Karst area. They are widely planted in southwestern China to harness rocky desertification due to its adaptability to the rock-desertification environment at Ca-rich, water and soil deficit karst areas (Yuan 2001). The report on the structure of leaf epidermis in Japanese honeysuckle and Wild Honeysuckle and their ecology adaptation is less, though many papers concerning quality control and evaluation of

Flos Loniceræ have been published (Xing 2003; Chai 2005). Leaf epidermal characteristics are of potential taxonomical importance (Jones 1986; Baronova 1992) and the leaf is more sensitive and plastic to environmental change than other organs because environment change usually results in morphological and anatomical responses of the leaf, including morphology (length, width, thickness), surface (stomata, epidermis, attachment) and mesophyll (palisade, spongy, intercellular space, sclerified, vein) (Wang *et al.* 1989). In present paper, we study the structure of leaf epidermis in Japanese honeysuckle and Wild Honeysuckle for classification in the future and recognize their ecological habits.

Material and methods

Plant material

Adult leaves of Japanese honeysuckle and Wild Honeysuckle prepared for this study were collected from the Nongla Karst Experimental Site, Institute of Karst Geology, Chinese Academy of Geological Sciences.

Structure of leaf epidermis

The adult leaves approximately at the same age were taken from fully expanded sun-exposed and cut into small blocks along the vein with 0.5 cm². The material for scanning electron microscopy (SEM) observation was macerated in FAA solution about 48 h, dehydrated in a series of gradient alcohol. Subsequently, the plant material was mounted on stubs with double-sided adhesive tape, and sputter coated with gold-palladium. After gold sputtering, the specimens were examined and photographed at 30 kV under a Quanta 200 scanning electron microscope at room

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temperature. The materials for light microscopy (LM) study were boiled in water before macerated in Jeffrey's solution (Stace 1965). Pieces of leaf epidermis were stained in a solution of 1% safranin (in 50% alcohol) before mounted in gum. To check the constancy of epidermal structure, three or more leaves were collected for each species. The stomatal index (SI) was calculated according to Meidner and Mansfield (1968):

$$SI = S / (E + S) \times 100\% \quad (1)$$

where, S is the number of stomata per unit area and E is the number of epidermal cells of the same area.

The classification proposed by Baranova (1987 1992) was followed in determining the type of stomata and the terminology of Wilkinson (1979) was adopted for other characters.

Table 1. The characteristics of epidermis cells

Taxa	Size of epidermal cells		Shapes of epidermal cell		Pattern of anticlinal walls	
	Upper	Lower	Upper	Lower	Upper	Lower
Japanese honeysuckle	70 μ m \times 46.5 μ m	48 μ m \times 25.8 μ m	Polygonal	Irregular	Straight	Sinuolate
Wild Honeysuckle	66 μ m \times 43.6 μ m	45 μ m \times 32.6 μ m	Polygonal	Irregular	Straight	Sinuolate

Table 2. The characteristics of stomatal apparatuses

Taxa	Size of stomata	Density of stomata	Stomatal index	Orientation of stomatal	Type of stomatal apparatuses
Japanese honeysuckle	23 μ m \times 21 μ m	420/mm ²	23.6%	Low epidermis	Anomocytic
Wild honeysuckle	22.5 μ m \times 20.5 μ m	328/mm ²	20.7%	Low epidermis	Anomocytic

Epidermal cells

Under SEM observation, the upper epidermis cells of Japanese honeysuckle and Wild Honeysuckle are polygonal and irregular in form, with anticlinal walls straight to arched, undulate or sinuous and periclinal walls depressed. Moreover, the boundary of the upper epidermis cells in Japanese honeysuckle and Wild Honeysuckle is very clear (Fig. 1 and 2).

The patterns of anticlinal walls on the low epidermis cells in Japanese honeysuckle and Wild Honeysuckle are different from the patterns on the upper leaf epidermis cells and the boundary of the low epidermis cells is not clear. The anticlinal wall on the low epidermis cells is undulate or sinuous, while the periclinal wall is depression and its surface is either smooth with shallow depressions or sculpturing with fine striats, or uneven with small projections on the lower epidermis cell of Japanese honeysuckle and Wild Honeysuckle (Fig. 9 and 10).

Moreover, by studying the leaf epidermal of the two species, it can be seen that the anticlinal walls on the upper epidermis in Japanese honeysuckle are diminutively undulate or sinuous and the leaf epidermal is undulate, while the anticlinal walls in Wild Honeysuckle are very obvious and its leaf epidermis are distributed by the tessellation character (Fig. 1 and 2).

Ttrichomes

The hair is distributed mainly on the lower leaf epidermis of the two species and belongs to glandular hair (Fig. 5, 6, 7 and 8). The epidermal hair on the lower leaf epidermis in Japanese honeysuckle is very dense and is mainly composed of glandular hair, and has little non-glandular hair (Fig. 5). While the epidermal hair on the lower leaf epidermis of Wild Honeysuckle is relatively sparse and can be classified into two kinds: glandular hair

The young and adult living-leaves for laser scanning confocal microscopy (LSCM) were washed with Hank's Balanced Salt Solution and loaded with Molecular Probes (Fluo-3/AM) made to 1 mmol/L and protected from light, at 4 °C for 2 h. And then, the samples were scanned by LSCM with the wavelength of 488 nm (Rijkers *et al.* 1990).

Results

The characteristics of leaf epidermis of *Flos Loniceræ* under LM observations are listed in Tables 1 and 2. It appears that stomatal and other epidermal features are constant within species and thus may be used in analysis and discussion.

and non-glandular hair (Fig. 6).

In addition, there are some glandular hair on the upper epidermis of Japanese honeysuckle arranged regularly along midrib, but Wild Honeysuckle has no glandular hair on the upper epidermis, which can provides some clues for classification (Fig. 3 and 4).

The head of glandular hair is spherical or hummocky with the diameter of 8–10 μ m and is composed of 4–10 cells. The petiole of the glandular is round and the total length of glandular hair changes from 100 μ m to 600 μ m. However, the non-glandular hair is composed of single sclerenchyma cells with the length of 70–800 μ m, the width of 6–60 μ m and the thickness of 2–20 μ m.

Stomatal apparatus

All species studied here are hypostomatic. The stomata may be paracytic, laterocytic, encyclocytic, incompletely encyclocytic, stephanocytic or less often anomocytic (Fig. 9 and 10).

The size of stomata was (22.5–27.5) μ m \times (14.5–22.5) μ m. In addition, the hair on the lower leaf epidermal of Japanese honeysuckle is very dense. Thereby, an airproof environment is formed to decrease water loss from stomata transpiration. However, the hair on the lower leaf epidermis in Wild Honeysuckle is relatively sparse, which have poor ability to decrease water loss from stomata.

Cuticular ornamentation

There are some equirostral strumae distributed around the stomata and arrayed in line on the lower leaf epidermis in Japanese honeysuckle and Wild Honeysuckle. The diameter of the strumae is ranging from 50 to 75 μ m.

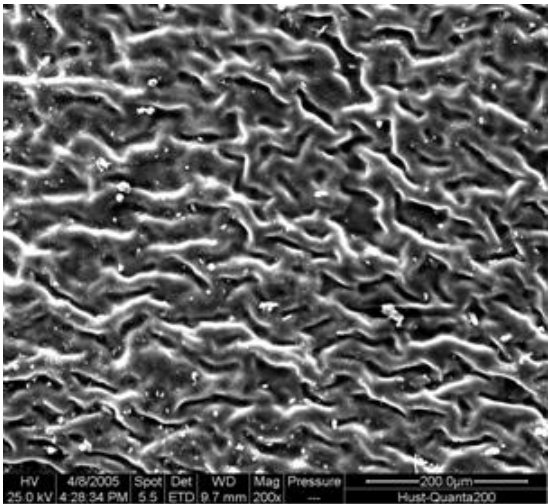


Fig. 1 Upper epidermis cells of Japanese honeysuckle,×200

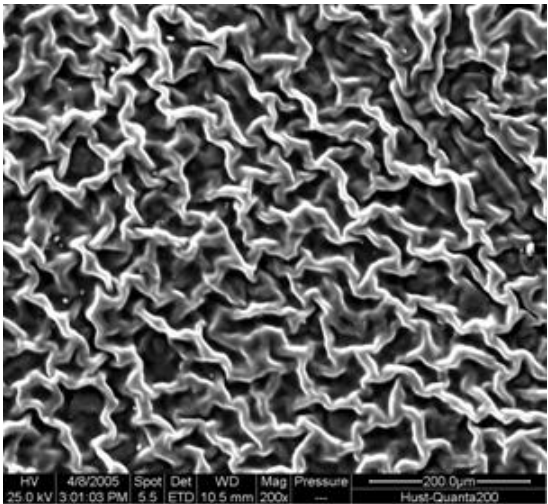


Fig. 2 Upper epidermis cells of Wild Honeysuckle,×200

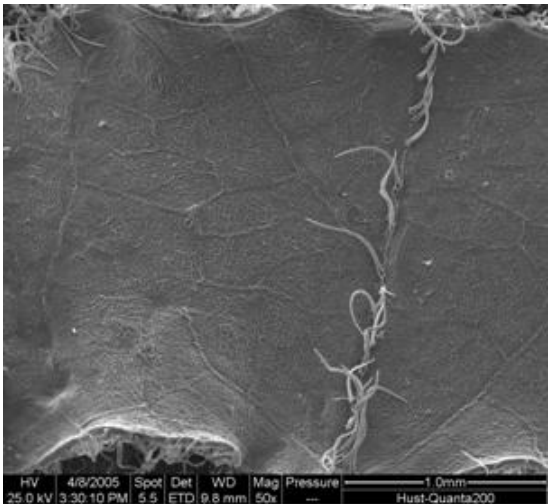


Fig. 3 Upper epidermis cells of Japanese honeysuckle,×50

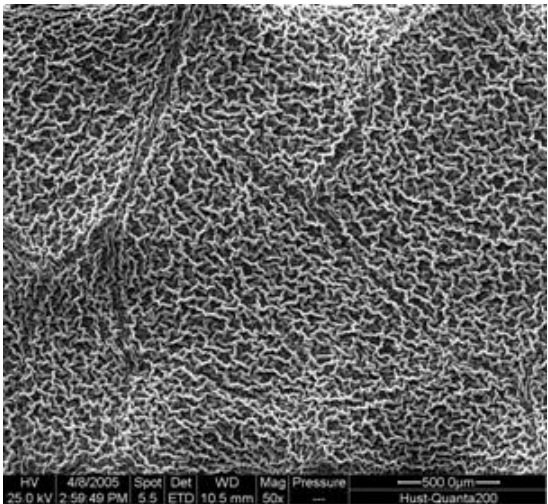


Fig. 4 Upper epidermis cells of Wild Honeysuckle,×50

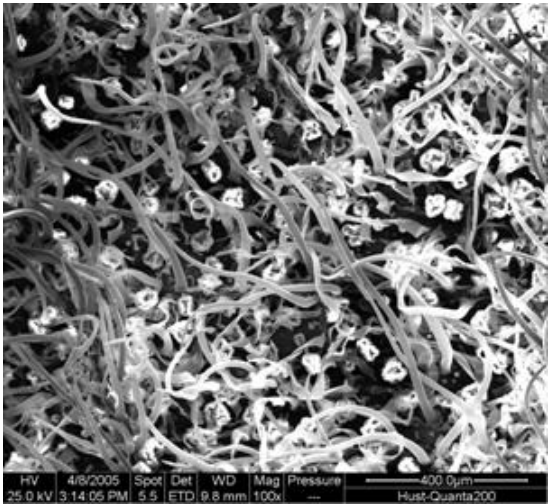


Fig. 5 Lower epidermis cells and trichomes of Japanese honeysuckle,×100

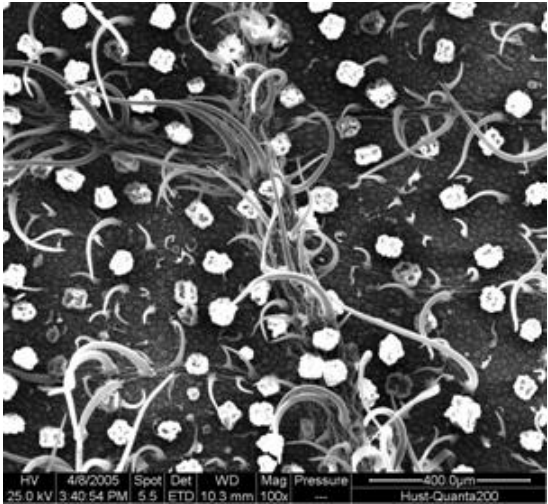


Fig. 6 Lower epidermis cells and trichomes of Wild Honey-suckle,×100

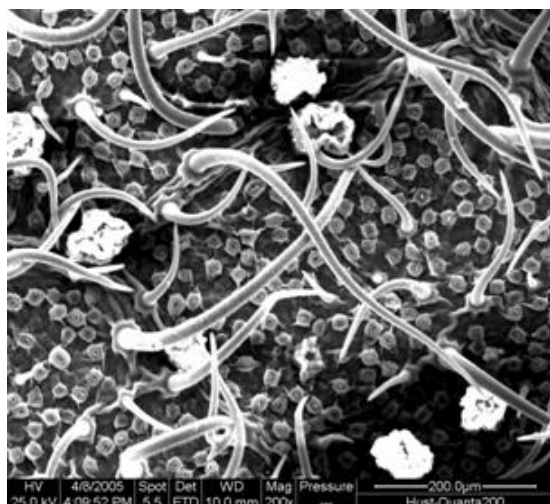


Fig. 7 Lower epidermis cells and trichomes of Japanese honeysuckle, ×1000

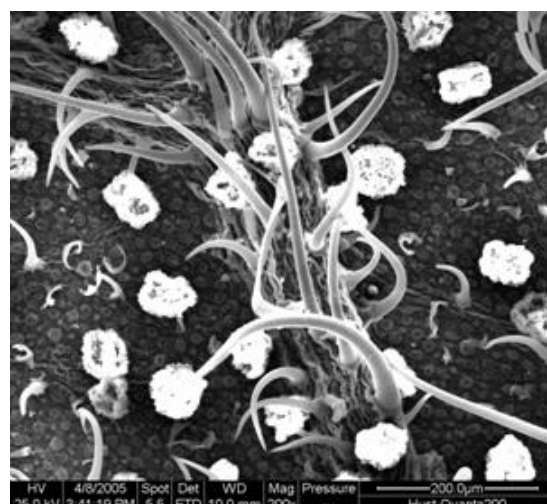


Fig. 8 Lower epidermis cells and trichomes of Wild Honeysuckle, ×1000

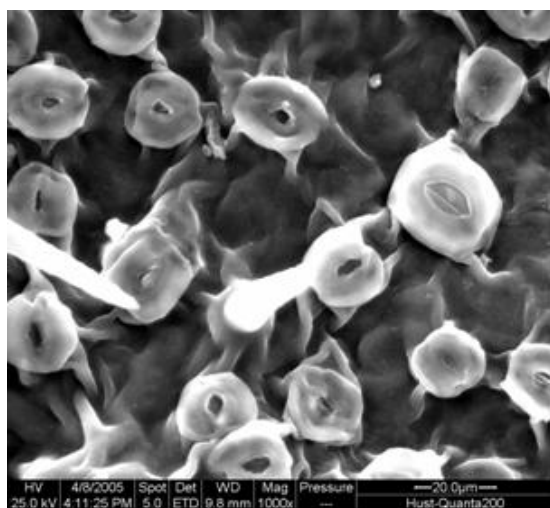


Fig. 9 Lower epidermis cells and stomata of Japanese honeysuckle, ×1000

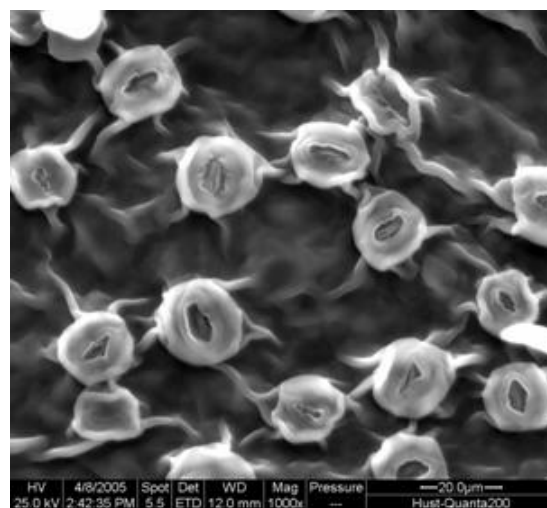


Fig. 10 Lower epidermis cells and stomata of Wild Honeysuckle, ×1000

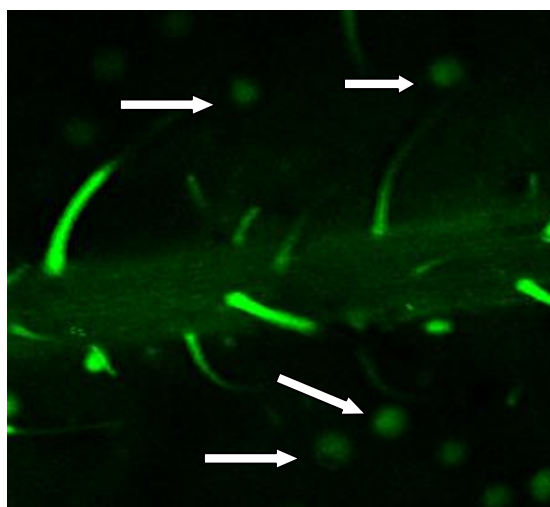


Fig. 11 Lower epidermis of adult leaf surface from Japanese honeysuckle dyed with Fluo3/AM

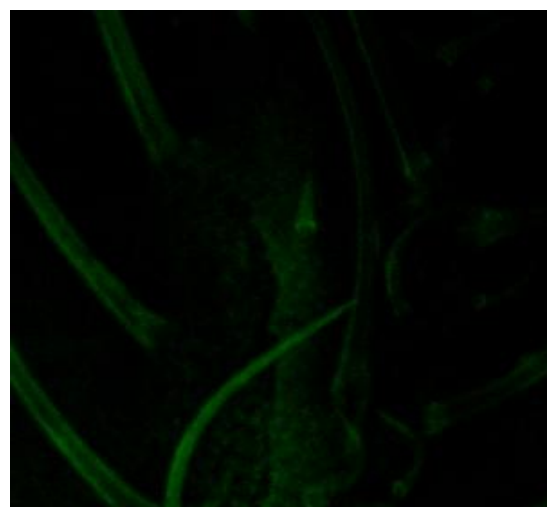


Fig. 12 Lower epidermis of young leaf surface from Japanese honeysuckle dyed with Fluo3/AM

By using x-ray to analyze the composition of the strumae, we found that the strume is composed of Ca, C and O (Fig. 13). To anatomize the strumae, the young and adult living-leaves from Japanese honeysuckle and Wild Honeysuckle loaded with Molecular Probes (Fluo-3/AM), were examined by LSCM with the wavelength of 488 nm. The concentration of intracellular Ca^{2+} is tightly regulated and change in Ca^{2+} flux released from intracellular stores or by influx of extracellular Ca^{2+} is a primary indicator of cell activation and function (Bootman *et al.* 2002; Parekh *et al.* 2005). Fluctuations and contents in Ca^{2+} can be monitored by several techniques with the selection being based upon factors including the stimulus and the required sensitivity of the measurement. Fluo-3/AM has the most commonly used fluorophore in flowcytometric Ca^{2+} measurements; it has an excitation peak at 488 nm, depending upon the Ca^{2+} concentration, and green fluorescence can be seen. The results showed that on the young lower-leaf epidermis there was no green fluorescence. However, there are some equirostral strumae with green fluorescence on the adult lower-leaf epidermis, which shows that the strumae can store the influx of extracellular Ca^{2+} (Figs. 11 and 12).

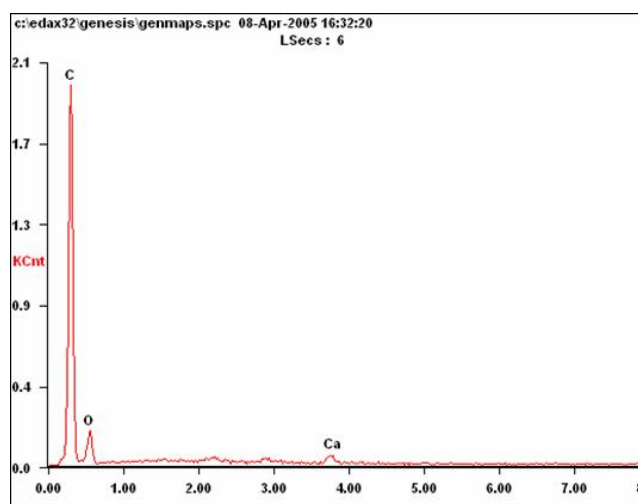


Fig. 13 The analysis of elements in the strumae

Discussion

In southwestern China, the karst ecosystem is fragile and is restrained by the karst environment, which is famous for soluble rock, soil scarcity, calcium-rich, alkaline and water leakage, and subterranean space, and rock desertification under the impact of anthropogenic activities is very serious. Therefore, precipitation can not be sufficiently assimilated by plant before rainfall runs out responding to the special karst flow system during rainy season, which causes the soil drought in the extreme. Accordingly, the karst ecosystem is featured as calciphile and xerophile on the surface (Yuan 1995; Yuan 2001). As an adaptive consequence, plants in the drought area developed various drought-resistant/tolerance mechanisms to adopt the drought stress environment, which are determined by environmental and genetic factors. Leaf as the biggest apparatus is exposed to the environment (Sheild 1950) and leaf epidermal characteristics are of potential taxonomical importance (Jones 1986; Baronova 1992).

The leaf morphology is affected in the extreme by the envi-

ronment. Thereby, studying the leaf morphology can realize its ecology adaptation on their leaf-structure such as well-developed epidermal hair, conducting tissue and mechanical tissue. For example, some plants adopt the protective ecotype to drought environment and have the well-developed epidermal hair and the waxlike, water-repellent cutin covering the epidermis, which can reduce transpiration and irradiation. Moreover, the kinds of plants also can decrease water loss when the stomata turn off timely (Fahn 1964; Johnosn 1975). Succulents have typically thick cuticles, a very low surface area to volume ratio and sunken stomata, which only open at night so as to minimize transpiration loss (Lyshede 1979). Some plants also take the abstemious ecotype to fit aridity. Geophytes effectively avoid periods of drought by surviving as underground corms or bulbs. Above ground parts are produced only after heavy rains. The resistant strategies is adopted by some plants, which have well-developed conducting tissue and mechanical tissue to supply enough water for live (Zhao *et al.* 1981; Wang *et al.* 1999).

In this study, the results showed that Japanese honeysuckle and Wild Honeysuckle have the xerophile characteristics, i. e. stomata, trichomes and dermal cell, *etc.* Under LM observations, Japanese honeysuckle and Wild Honeysuckle have high density stomata and low stomatal index. Farquhar and Sharkey (1982) pointed out that stomata control the carbon-water balance in plants by acting as transport for diffusive CO_2 uptake and water vapor loss. Drought-resistant species should have high density stomata, which maximize the rate of carbon gain while minimizing water loss (Sam *et al.* 2000; Bosabalidis *et al.* 2002). Under SEM observation, the stomata apparatus are ruleless and are distributed only on the lower epidermis in Japanese honeysuckle and Wild Honeysuckle. And, the upper epidermis cells in Japanese honeysuckle and Wild Honeysuckle are polygonal and irregular in form, with anticlinal walls straight to arched, undulate or sinuous and periclinal walls depressed. Moreover, on the lower epidermis, bushy trichomes including glandular hair and non-glandular hair are observed. Well-developed epidermal hair and depressed periclinal walls can minimize transpiration (Ferris 1996). So, we could infer that Japanese honeysuckle and Wild Honeysuckle adopt the water saver-ecotype to drought karst environment. Moreover, it can be concluded that Japanese honeysuckle has the stronger drought-resistance and uses water more efficiently than Wild Honeysuckle from the leaf epidermal feature because Japanese honeysuckle has more epidermal hair on the lower epidermis, which forms a relatively airproof space and reduces water loss from stomata under severe water deficit, and is efficient water use types.

Under SEM and LSCM observation, some strumae distributing are regularly hypothesized as oxalic calcium on the lower epidermis in Japanese honeysuckle and Wild Honeysuckle. At karst area, the soil is famous for calcium-rich, alkaline (Yuan 2001). So, Ca^{2+} from the soil will be absorbed incessantly by plants under the transpiration power. The accumulation of Ca^{2+} in the living organism of plant will disturb the physiological function and bring injure to the apparatus (Du *et al.* 1995). In order to avoid the ion damage, the plant will form some strumae to store the ion released from intracellular stores or extracellular influx, which can improve the plants' ability to drought stress (Kausik *et al.* 1977). From the SEM, x-ray and LSCM results, we could infer that Japanese honeysuckle and Wild Honeysuckle at karst area produce glandulose strumae adjacency to the stomata to store Ca^{2+} and to promote the drought stress. Some authors think that the strumae is the outgrowth of the leaf trans-

ported through the leaf surface or the secretion of secretory, which has the function to protect the leaf (Pant *et al.* 1977). Moreover, some authors think that the strumae is the fasciation crystal of calcium oxalate, which can improve the plants' ability to drought stress (Kausik *et al.* 1977; Kausch *et al.* 1981).

Moreover, there is some difference in Japanese honeysuckle and Wild Honeysuckle by comparing the leaf epidermal feature. From the SEM result, it is shown that on the upper epidermis, some glandular hair regularly present along the midrib of Japanese honeysuckle, while Wild Honeysuckle has no glandular hair on the upper epidermis, which can provide the significance for classification of *Flos Loniceræ*.

From the above analysis, we could conclude that Japanese honeysuckle and Wild Honeysuckle had the xerophile character to drought stress. The previous fieldwork also declared that *Flos Loniceræ* can tolerance drought when water moisture is deficit (Yuan 2001). Moreover, the flower from *Flos Loniceræ* has important function. Thereby, planting *Flos Loniceræ* at karst area can not only improve the incoming of the local people in southwestern China, but also harness efficiently water and soil loss, and rock desertification.

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